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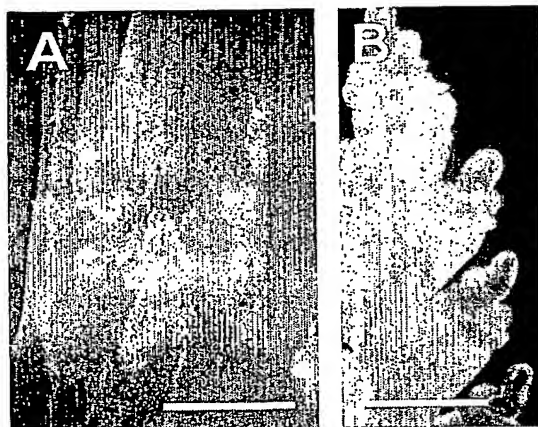
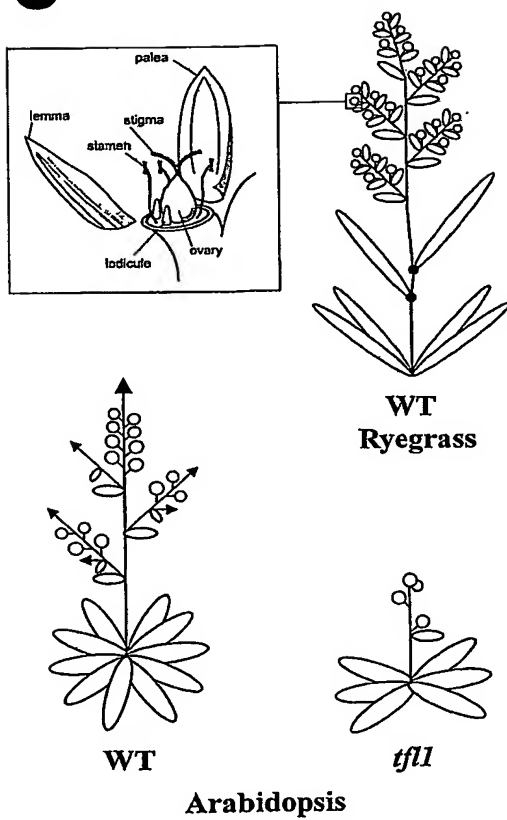
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- (74) Agents: **MACDOUGALL, Donald, Carmichael et al.**; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).
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- (71) Applicants (*for all designated States except US*): **DLF - TRIFOLIUM A/S** [DK/DK]; Hoejerupvej, P.O. Box 19, DK-4660 Store Hedding (DK). **RISØE NATIONAL LABORATORY** [DK/DK]; P.O. Box 49, DK-4000 Roskilde (DK).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **NIELSEN, Klaus, K.** [DK/DK]; Nebbegaardsbakken 9, DK-2400 Copenhagen NV (DK). **JENSEN, Christian, Sig** [DK/DK]; Moltkesvej 61, 2th, DK-2000 Frederiksberg (DK). **GAO, Caixa** [DK/DK]; Maglekaeret 37B, DK-2680 Solroed Strand (DK). **SALCHERT, Klaus** [DE/DE]; Steinberg Strasse 16, 06507 Gernrode (DE).
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(54) Title: METHOD OF REPRESSING FLOWERING IN A PLANT

(57) Abstract: The isolation and function of a plant *LpTFL1* from *Lolium perenne* (perennial ryegrass) are described, along with generation of transgenic *Arabidopsis*, ryegrass, and red fescue plants. The gene prevents or represses flowering of transgenic plants. Methods for using the gene to repress or prevent flowering are described.

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FIGURE 1**C**

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FIGURE 2

		GCC	-76
-75	CAAGCCACTTCAAAGCTTTGCTACTACCAGATAGAGCATTACCGTGCAATATAGAAATACTTGCCTCTCCAACC		-1
1	ATGTCTAGGTCTGTGGAGCCTCTTATTGTTGGTCGTGTTCATTGGAGAAGTTCTCGATCCATTTAACCCATGTGTG		75
76	AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCATGAGCTCTACCCATCAGCAGTTGTATCT		150
151	AAACCAAGAGTAGAGGTTGAGGGGGGTGACTTGCGATCCTTATTCACATTGGTTATGACGGACCCAGATGTGCCA		225
226	GGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGATTGTCAGTAATATACCTGGGACAACAGATGCTTCA		300
301	TTTGGGGGGGAGGTCATGAGCTATGAGAGCCCAAAGCCCAACATTGGAATCCACAGGTTCATTTTTGTGCTCTTC		375
375	AAGCAGAAGCGAAGGCAGACTGTATCTGTGCCTTCCTTCAGGGATCATTTCAACACCCGCCAGTTTGCTGTGGAT		450
451	AATGATCTTGGCCTCCCTGTGGCTGCTGTTTACTTCAATTGTGAGAGAGAGACTGCTGCCAGGAGGCGCTGAAAA		525
526	TCGAGTTCTTGGCTATCCAGTTGTGCCAAATAAAGGCTTTTGAGTTATGCACCTTCTTTCTGAAGTCAATGCT		600
601	CCTCTTCTACATTACTTCCTCGTGGACCATTGCTTCTTTACTACAGTTTTTGCTCAGGGATCAAATAAATCAAGT		675
675	GCATTTTGGAGATTGTATTAGATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTTAACATAAGCCAGTACAT		750
751	TAGCAGGTCCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAGGTCAGGTAGACAACCCA		825
826	AACTGGCAAAAAAAGCTTTATCTA		851

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FIGURE 3

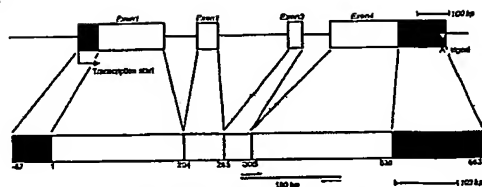
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-3000 aatactatagaacttccataatttaatttccccttattttctggactctatcttaattctcctcctattgttcag -2926
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-2850 cctgaatctatttctcactcatgctgcaatgctccttctcacagcaaatatgggtatgatctgcagtaagctc -2776
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-2700 ccagccctgcagaaggggtgctaaatccatcatcatccttactctctgagattatggaagacgaggaacgatgct -2626
-2625 atcttcaaaaatctggccccaacagactgccttagttcagtcgatcctagatgaagcctgtcaatggctgta -2551
-2550 gccgggtgctaagcgctacgtcagttacctttacatgcttagacccctgatgttagccttgatgaggaactctag -2476
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-2250 aatcgcaacttgtccaagctgctgcgcgtggtgctgctgtccttttccaatgcatccatcactgtcctagtcag -2176
-2175 cataccaaaaaaaagctaatgcccgcctgtgtttcacaatgaattatctgattgtgatgctgctaactctttt -2101
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-2025 cactggtaagctgaagttctgtgagcaggctatgatttcccctgttaaaaaaaggctatgaaaaacttgtgat -1951
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-1875 ctactgtgtctaccaacacactgtagcactgccaaaaatttatgaaaaagctgtaacagacgagatgtatctatca -1801
-1800 attcatggaccattttgttataattttcttttaaaaaataaaaaatccgtaaaagaatcaataagtggaattattg -1726
-1725 gaaatgaaaaaagtaacaaaaataactaaacttttttcaaatacagatcggtatcatggagacacactggctac -1651
-1650 cattggttgaatagctactagattccactacagctaggtgtcagcaactataatggcatcagaatggagcaga -1576
-1575 aaaaatgtcacagctgtacttccactcactcttctagctgcacaaatgtcaagcaggcatgattgcactagacc -1501
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-1425 aatcgagatgaagctgtgataattttatcgctgaaatgacatttcagcactagacagaccctagacaattaaatg -1351
-1350 ggtggtggcactgtattccattcctttattctcttccatgggtgtgttcccatagtagtactacaagaagagataaa -1276
-1275 cagataataatggtaatgcacttgggtatcgaagtttttaggaaagattctaattctagagcaattgaaactcaaca -1201
-1200 acaacttcccttttcttaacagaaaaagaatcggtcacaacgaggttgcctaaacacaaactataaagacg -1126
-1125 aacatttgagggtgaagaggcttccacgtggacagctgcccagctgtttctgtccactagataaacacctaataata -1051
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-825 cattgttctgtaattgtggctgtgaatttcggactgctcatctgatttccctctggtagaatacataaataattat -751
-750 acacaacagcatgataatgtgcaaaactaagcatcaaaatctgcacattgtcatgcagaaactaggacaggagga -676
-675 ccagcactttgtcgtttgtcctaaccaatattaacatagttcagcaacataatcttcagagaccactagcatga -601
-600 aggtgtgttatgtttcctaagaataaacatgtaggtagtgatctacaatacctttttggggactataaggtgg -526
-525 gaaaccatcaacttgaaaagggttccatttaataagtaaaaaaacagattttttaactatcaataactaaaa -451
-450 ttaaaacagataagagatataactaacaatgaaaatcaaacagttgtgcaaatgtatttatcgtagttagtatct -376
-375 catgtttctggtgaaaaaattctctgcccctagaacttggaagaagatgcatgaagtattactccaaactccaac -301
-300 actgtgcaactgatagaaaagaacaagacccttggttggctgtctcggaagagtggttaggtcctttctgtgg -226
-225 ccttttcagttctttccacgcataaccaacaaaaagaacacagataactactcatgtctcacattctcttttga -151
-150 gcttacactcgaagcaggcttcttgcctctataagtagaggctcgctgactctagcaatgctcagtaagcaGCC -76
-75 CAAGCCACTTCAAAGCTTTGCTACTACAGATAGAGCATTCACCGTGCAATATAGAAATACTTGCCCTCTCCAACC -1
1 ATGCTCTAGGCTCTGTGGAGCCTCTTATTGTTGGTCTGTCTATTGGAGAAGTTCTCGATCCATTTAAGCCATGTGTG 75
76 AAGATGGTAGCAACCTATAACTCAAACAAGCTGGTCTTCAATGGTCAAGAGCTCTACCCATCAGCAGTTGTATCT 150
151 AAACCAAGTAGAGGTTTCAAGGGGGGTGACTTGCGATCCTTATTACATTGGtagaatgcactcgactcgatctt 225
226 ggaactcatattcaacttcgagattgtatgcttgttttcttcttctcgcagtgccataattattcatatttca 300
301 gTTTATGACGGACCCAGATGTGCCAGGACCAAGTGATCCGTATCTGCGGGAGCATCTTCACTGGTaaacctttctc 375
375 atgcacagtttttctgctgggtggtactaagcacctaaatatattagatatattttttgaaaggaaaatatat 450

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451	tagtatatgttgctaaggaatatagaagtacatcttcttcttgccacatatatagacagagagactattttaatatag	525
526	cacttctaacgagaggtcattttaccaataccttttacacttacacaggATTGTCAGTAATATACCTGGGACAACAG	600
601	ATGCTTCATTGGtaggtccttctctgagatttgaattgggtatattctatgttctgcattttgaatgaataacca	675
675	ctgaccttttgaattgcaggGGGGGAGGTCATGAGCTATGAGAGCCCCAAGCCCAACATTGGAATCCACAGGTTTC	750
751	ATTTTGTGCTCTTCAAGCAGAAGCGAAGGCAGACTGTATCTGTGCCCTTCCTTCAGGGATCATTTCAACACCCGC	825
826	CAGTTTGTGCTGGATAATGATCTTGGCCTCCCTGTGGCTGCTGTTTACTTCAATTGTCAGAGAGAGACTGCTGCC	900
901	AGGAGGCGCTGAAAATCGAGTTCTTGGCTATCCAGTTGTGCCAAATAAAGGCTTTTGGAGTTATGCACCTTCTT	975
976	TCTGAAGTCAATGCTCCTCTTCTACATTACTTCCTCGTGGACCATTTGCTTCTTTACTACAGTTTTTGTCTCAGGGA	1050
1051	TCAAATAAATCAAGTGCATTTTGGAGATTGTATTAGATTATATTGTAAGCAGTGAGATCAGCAACCATGTGTAA	1125
1126	CATAAGCCAGTACATTAGCAGGTCCATGTTTATGGTTTCATGTTGTGTGTAAGCAGTTATCACTAGAAGGAAGGT	1200
1201	CAGGTAGACAACCCAAACTGGCAAAAAAAAAAGCTTTATCTActgtatggcccttgccggttgatgttccatgc	1275
1276	accttttctgacatgctgtctactgtatgccaccgccactataatgtatgagatatgaatataaaatggagatat	1350
1351	ccaaaatatccagatgattgccactaaatgctaaatgtacatagtggttttccacctattttgacttcatcat	1425
1426	gtccttacacaaaatcagaaaacatccatttcatgcacattgatgcacactgcatattaacaatctattcagatt	1500
1501	tggctgtaaacacaccttattttccgcattccattaatattatattagtagtaccctggacaggttaagcttttgcag	1575
1576	cacagtaagtaaccggatgaaattacaatatgatcctcgagcgccctat	1624

FIGURE 4

1	MSRSVEPLIVGRVIGEVLDPFNPCKMVAATYNSNKLVFNGHELYPSAVVSKPRVEVQGGDLRLSLFTLVMTDPDVP	75
76	GPSDPYLRHLHWIVSNIPGTTDASFGGEVMSYESPKPNIGIHRFIFVLFKQRRQTIVSVPSFRDHFNTNRQFAVD	150
151	NDLGLPVAAVYFNCQRETAARRR	173

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FIGURE 5**A****B**

TFL1	M	N	M	G	R	V	I	E	P	L	M	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	S	H	L	37
BNTFL1-1	M	N	M	G	R	V	I	E	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	S	H	L	37
BNTFL1-3	M	N	M	G	R	V	I	E	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	S	H	L	37
CEN	M	-	-	-	A	A	K	V	S	S	D	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	35
CET2	M	-	-	-	G	S	K	M	S	-	D	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	34
CET4	M	-	-	-	G	S	K	M	S	-	D	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	34
SP	M	-	-	-	A	A	K	V	S	S	D	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	34
CET1	M	-	-	-	A	A	K	V	S	S	D	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	34
LpTFL1	M	-	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	33
FDR2	M	-	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	33
FDR1	M	-	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	33
FT	M	-	-	-	S	R	S	V	-	-	E	P	L	V	G	R	V	V	G	D	V	L	D	F	T	F	T	K	H	N	V	34

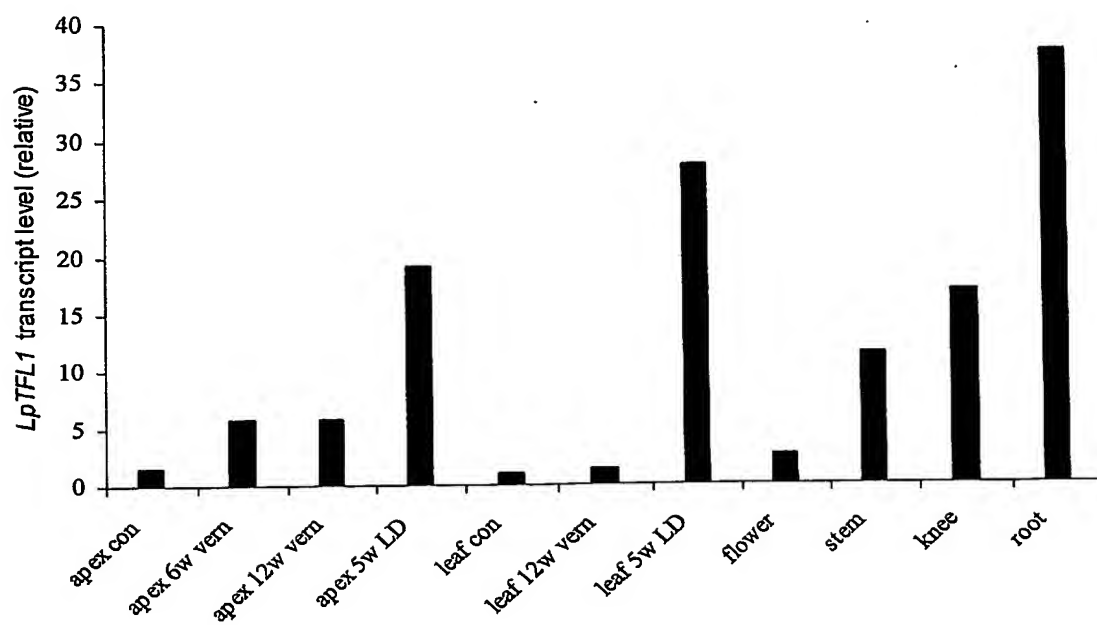
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BNTFL1-1	-	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	71
BNTFL1-3	-	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	71
CEN	M	N	S	K	M	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	73	
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CET4	S	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	69
SP	S	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	69
CET1	S	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	69
LpTFL1	N	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	68
FDR2	N	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	68
FDR1	N	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	68
FT	N	-	-	-	K	Q	V	S	H	G	H	E	L	F	P	S	A	V	S	K	P	R	V	E	S	G	G	L	R	S	F	T	L	V	68

TFL1	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	108
BNTFL1-1	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	109
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CEN	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	111
CET2	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
CET4	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
SP	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
CET1	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	107
LpTFL1	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106
FDR2	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106
FDR1	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106
FT	M	N	D	P	D	V	G	P	S	D	F	L	R	E	H	L	H	V	I	T	D	I	P	G	T	D	A	S	F	G	R	E	V	106

TFL1	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	145	
BNTFL1-1	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	146	
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CEN	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	149	
CET2	G	T	E	M	F	R	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	143
CET4	G	T	E	M	F	R	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	143
SP	G	T	E	M	F	R	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	143
CET1	G	T	E	M	F	R	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	142
LpTFL1	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	141	
FDR2	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	141	
FDR1	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	141	
FT	S	T	K	P	K	N	I	G	H	R	F	V	V	L	F	R	Q	R	-	R	V	I	F	-	H	I	P	S	R	D	H	141	

TFL1	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	177
BNTFL1-1	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	178
BNTFL1-3	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	178
CEN	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	181
CET2	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	175
CET4	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	175
SP	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	175
CET1	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	174
LpTFL1	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	173
FDR2	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	173
FDR1	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	173
FT	F	H	T	R	F	A	E	H	D	L	G	L	P	V	A	A	V	F	F	H	A	Q	R	E	T	A	-	-	-	-	-	-	175

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FIGURE 6

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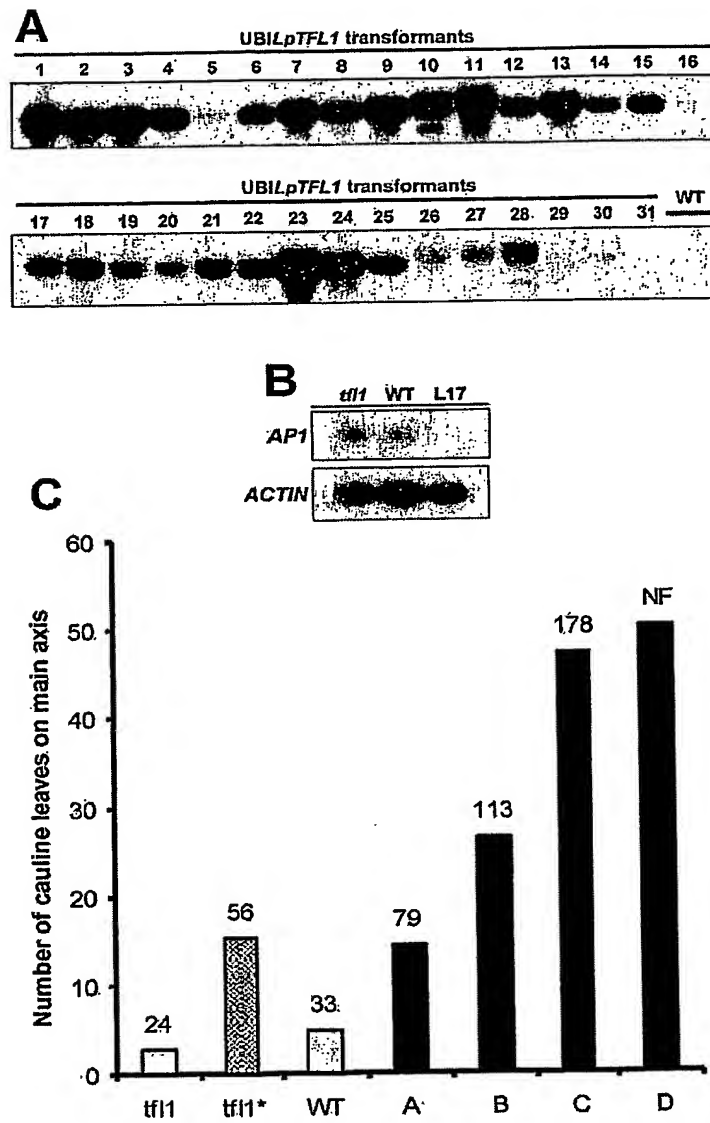
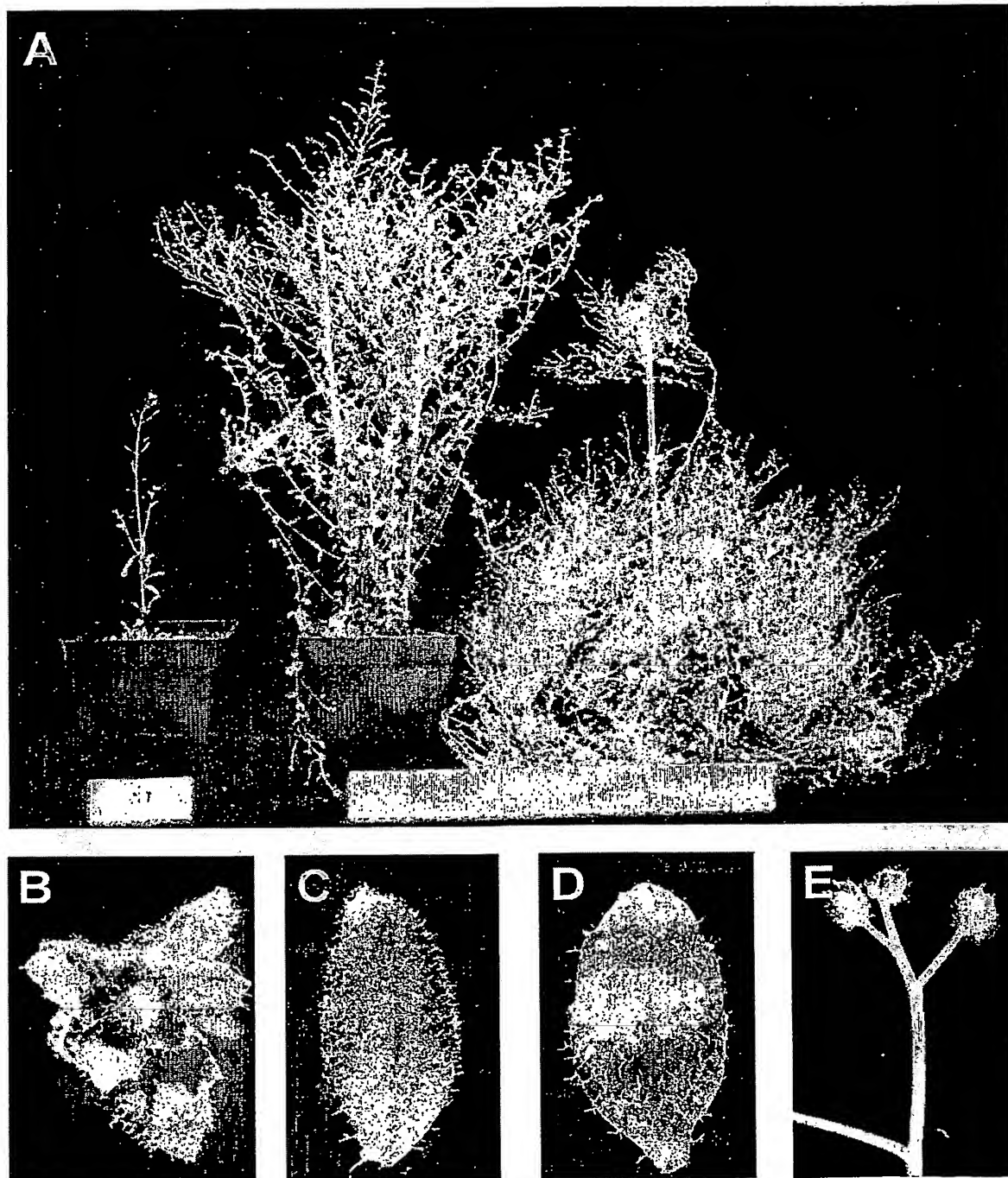
FIGURE 7

FIGURE 8



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FIGURE 9

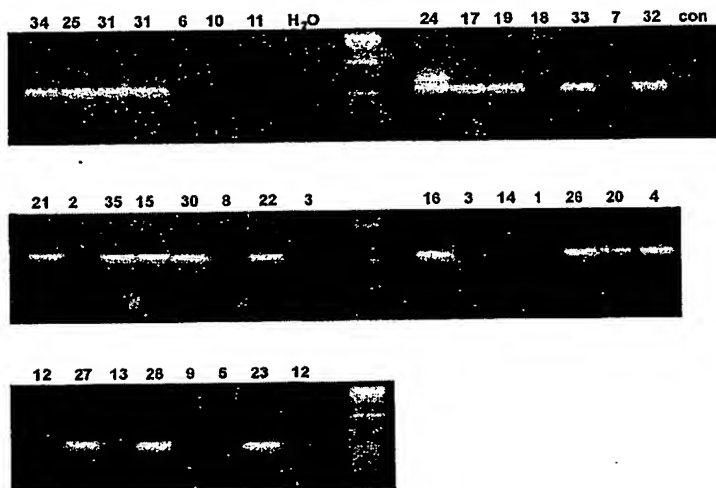


FIGURE 10

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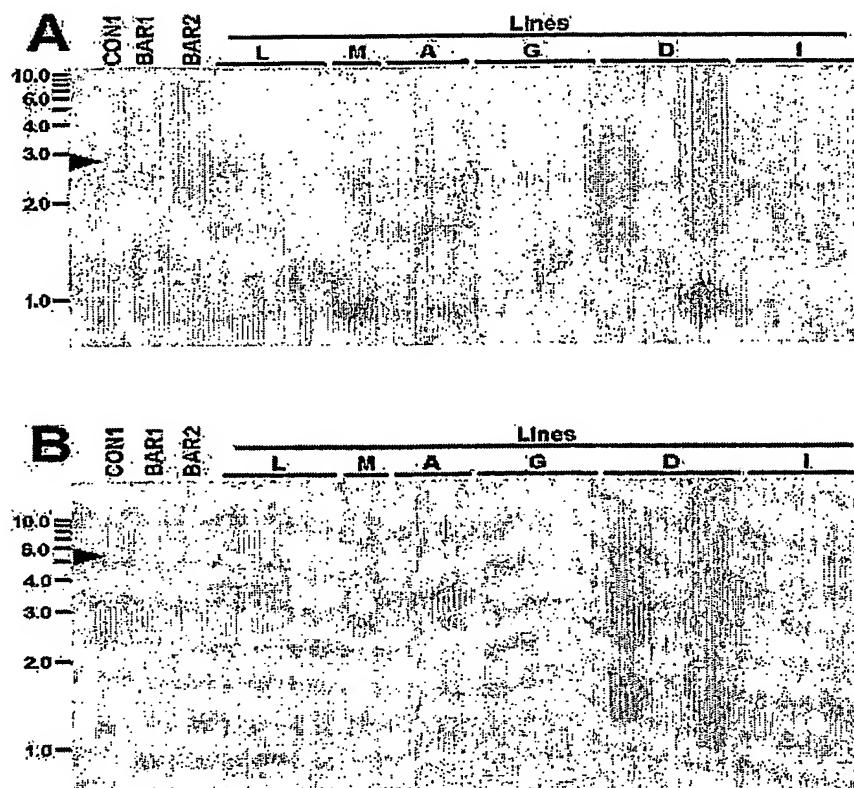
FIGURE 11

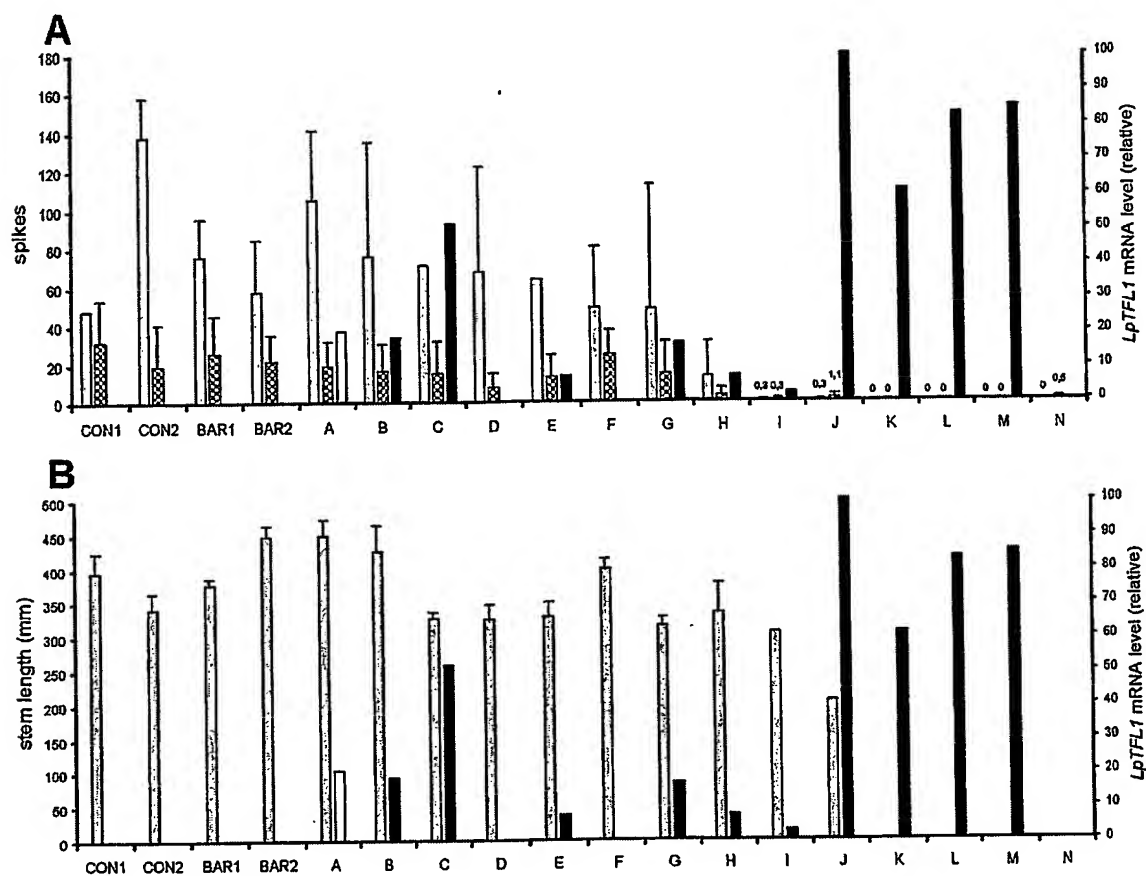
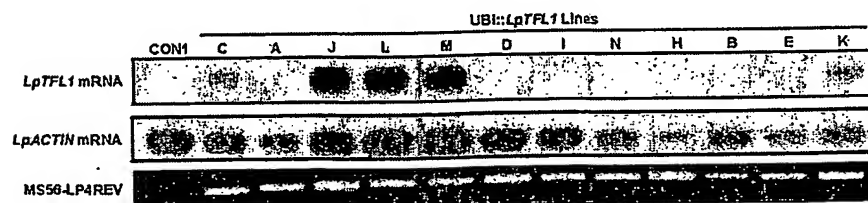
FIGURE 12**FIGURE 13**

FIGURE 14

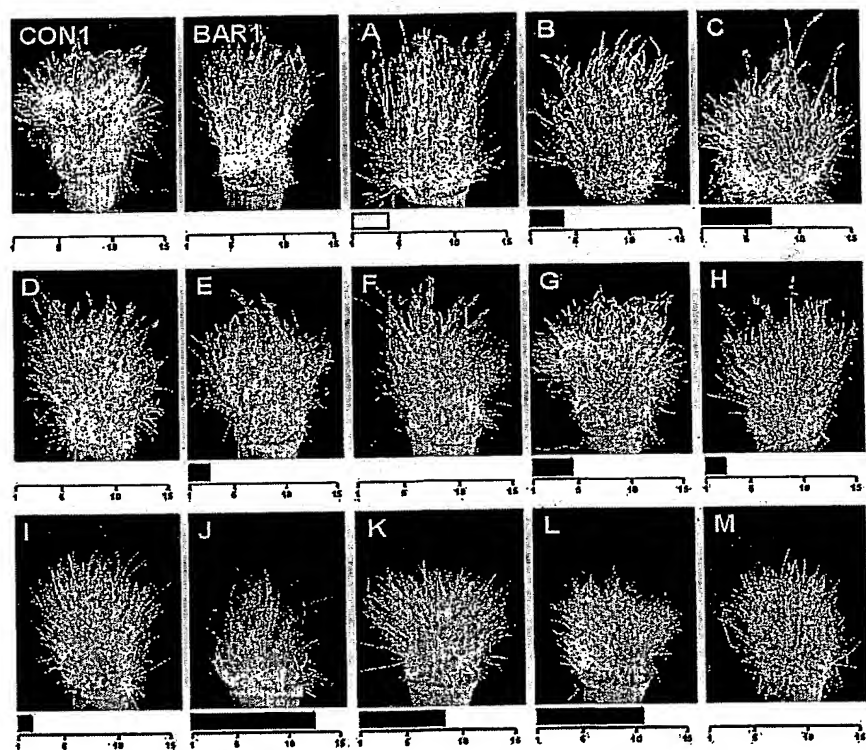


FIGURE 15

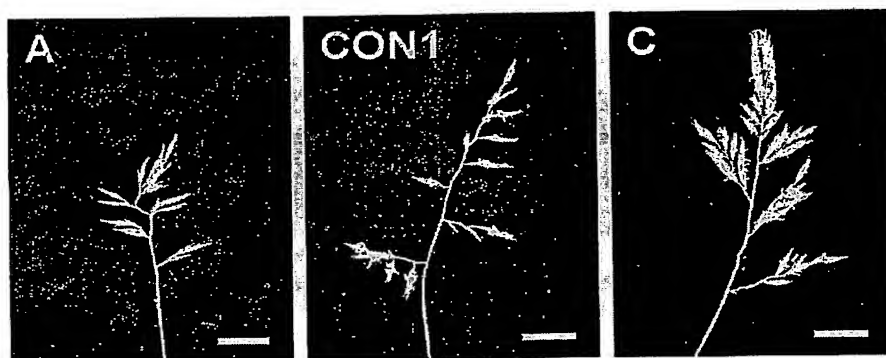
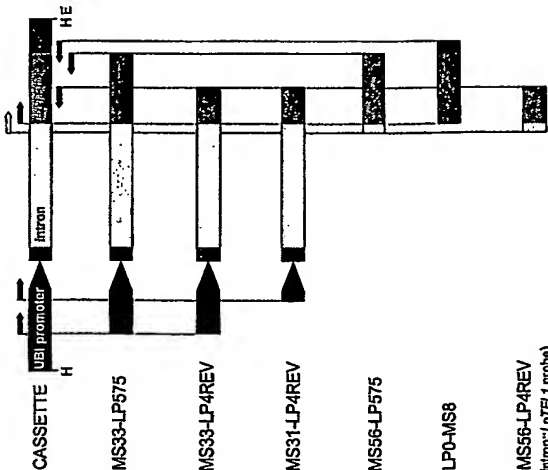
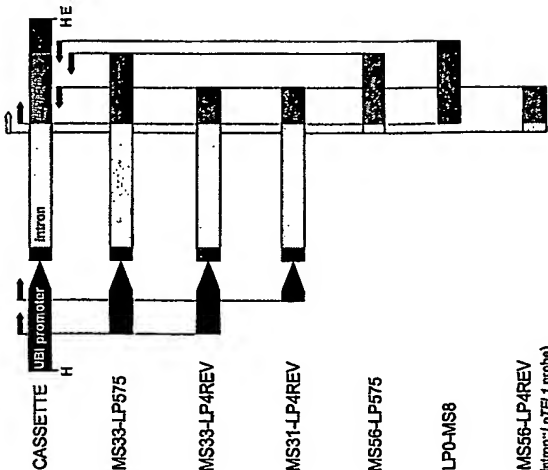
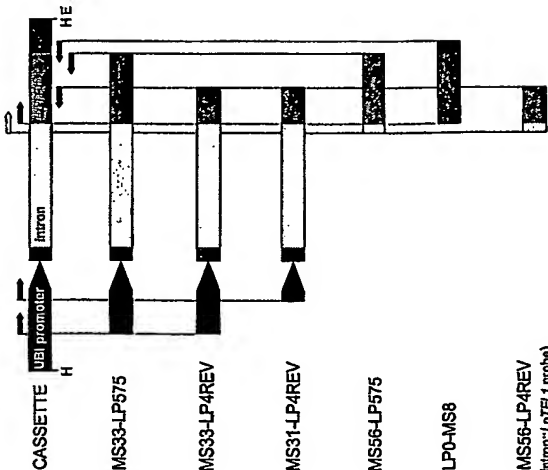
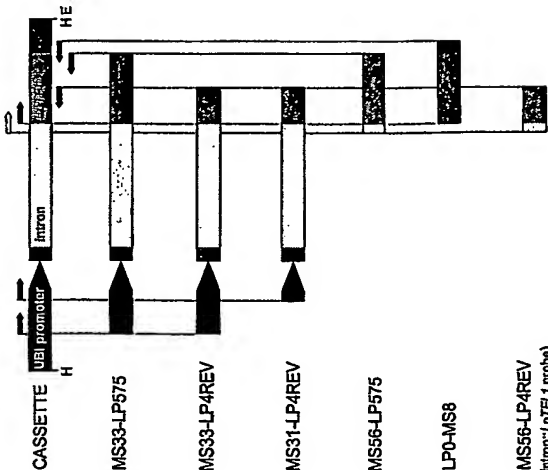
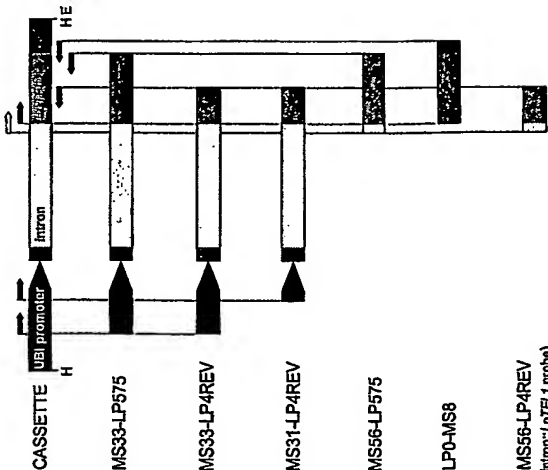
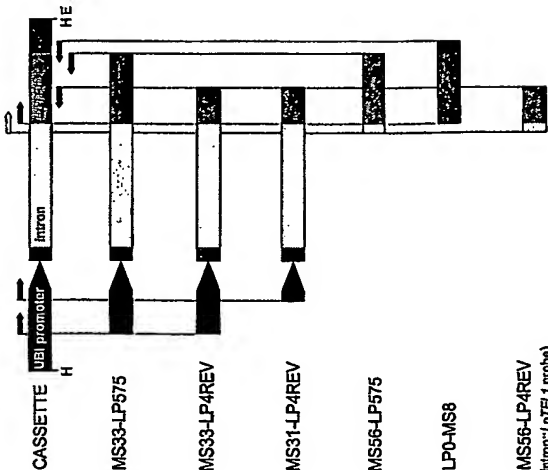


FIGURE 16: *Transformation Efficiency and Floral Activity of the Transformants*

Cultivar	Line No.	Inflorescences	PCR	RT-PCR
F6	CON	8	-	-
F6	7	18	-	-
F6	8	11	-	-
F6	17	5,3	+	-
F6	18	13,3	+	-
F6	24	12	+	+
F6	29	0	+	+
F6	32	0	+	+
F6	33	4	+	+
F6	36	0	+	+
ACTION	2	1,8	-	-
ACTION	5	3	-	-
ACTION	9	0,3	-	-
ACTION	12	2	-	-
ACTION	13	0	-	-
ACTION	16	0	+	-
ACTION	19	7,3	+	-
ACTION	21	4	+	+
ACTION	22	0,3	+	+
ACTION	23	0	+	+
ACTION	25	0,3	+	+
ACTION	27	0	+	+
ACTION	28	4	+	+
ACTION	31	0	+	+
ACTION	34	0	+	+
ACTION	35	0	+	+
TELSTAR	1	10	-	-
TELSTAR	3	1	-	-
TELSTAR	4	11,6	-	-
TELSTAR	6	10,8	-	-
TELSTAR	10	5	-	-
TELSTAR	11	3,8	-	-
TELSTAR	14	0	-	-
TELSTAR	15	3,8	+	-
TELSTAR	20	3,5	+	-
TELSTAR	26	0	+	+
TELSTAR	30	3,7	+	+

Figur 17: Transgene integration analysis by PCR using different primer combinations

Primer combination	UBI::LpTFL1 transgenic lines ^a																				
	CASSETTE	CON	BAR	A	B	C	D	E	F	G	H	I	J	K	L	M	N	P			
MS33-LP575							0.8					+	+		+		0.8	2.3			
MS33-LP4REV							0.55					+	+		+		0.55	2.0			
MS31-LP4REV		+					+			1.4	+	+	+		+	+		1.5			
MS56-LP575			+	+	+	+	+0.5	+		+0.5	+	+	+	+	+	+	+0.5	0.6			
LP0-MS8			+	+	+	+	+M	+		+	+	+1.8	+	+	+	+	+1.6	0.6			
MS56-LP4REV (identical to LpTFL1 probe)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.4			
Result	Promoter	short					TATA box			short	short	ok	ok	ok	ok	short	TATA box				
Intron		ok					ok			ok	ok	ok	ok	ok	ok	ok	ok				
LpTFL1 cDNA		truncated	ok	ok	ok	ok	ok + extra	ok	truncated	ok	ok	ok + extra	ok	ok	ok	ok	ok + extra				

^aplus indicates that the observed fragment had the expected size, whereas numbers indicate that the fragment size deviated from the expected size (numbers in bold), blank field indicates that no PCR-product was detected; E, *EcoRI*; H, *HindIII*

^aplus indicates that the observed fragment had the expected size, whereas numbers indicate that the fragment size deviated from the expected size (numbers in bold), blank field indicates that no PCR-product was detected; E, *EcoRI*; H, *HindIII*